measured, as shown in Table I. Since these four compounds have similar UV absorption characteristics, the rates of polymerization should provide an approximate indication of relative radical yields and reactivity. In aqueous solution the values of the rates suggest that cation radical production does not depend upon loss of chloride, as evidenced by the comparatively high rate sensitized by IIIa. However, in methanol solution the three compounds that lose chlorine show about the same rate of polymerization, a higher value than that for IIIa.

On the basis of the above results, the primary photochemical processes occurring on irradiation of hydrochlorothiazide appear similar to those found for chlorpromazine, with photoionization predominating in aqueous solution and triplet state formation mainly in methanol. However, it is possible that the polymerization technique is not an adequate indicator of radical ion formation in methanol where ion recombination or reaction with solvent may occur more readily than in water. A phenomenon that is indicative of excited state electron transfer is the quenching of fluorescence by an electron donor (17). We have observed that the fluorescence of I (50 μM in methanol) is quenched to 50% by 6 mM triethylamine. In contrast, the fluorescence of chlorpromazine is unaffected (18).

The fact that hydrolysis of I is stimulated by irradiation implies the formation of a cation radical in the thiadiazine ring, thereby rendering it more susceptible to attack by nucleophiles. This is most evident in the aqueous system where the expected intermediate IIIb was not detected. The dechlorination is presumably effected by the solvated electron formed in the photoionization.

In methanol the formation of cation radicals is indicated by the presence of significant amounts of the hydrolyzed compound II. However it is not clear if the cation radicals result from photoionization or radical-ion-pair formation from the triplet state. Flash photolysis experiments are needed to clarify the primary photochemical events. However, as pointed out elsewhere (10, 16), it is believed that the formation of free radicals following absorption of UV radiation is the significant factor in the initiation of a photobiological effect.

REFERENCES

(1) I. A. Magnus, "Dermatological Photobiology," Blackwell, Oxford, 1976, pp. 213–215.

(2) D. E. Moore, *J. Pharm. Sci.*, **66,** 1282 (1977).

(3) D. E. Moore and S. R. Tamat, *J. Pharm. Pharmacol.*, **32,** 172 (1980).

(4) J. G. Jose, *Proc. Natl. Acad. Sci. USA*, **76,** 469 (1979).

(5) F. C. Novello and J. M. Sprague, *J. Am. Chem. Soc.*, **79,** 2028 (1957).

(6) L. H. Werner, A. Halamandaris, S. Ricca, L. Dorfman, and G. de Stevens, *J. Am. Chem. Soc.*, **82,** 1161 (1960).

(7) W. J. A. Vandenheuvel, *J. Pharm. Sci.*, **64,** 1309 (1975).

(8) J. P. Soumillion and B. De Wolf, *J. Chem. Soc. Chem. Commun.*, **1981,** 436.

(9) C. R. Rehm and J. B. Smith, *J. Am. Pharm. Assoc., Sci. Ed.*, **49,** 386 (1960).

(10) A. K. Davies, S. Navaratnam, and G. O. Phillips, *J. Chem. Soc. Perkin Trans. 2*, **1976,** 25.

(11) H. Barton, J. Mokrosz, J. Bojarski, and M. Klimczak, *Pharmazie*, **35,** 155 (1980).

(12) R. Davis, C. H. J. Wells, and A. R. Taylor, *J. Pharm. Sci.*, **68,** 1063 (1979).

(13) N. J. Bunce and L. Ravanal, *J. Am. Chem. Soc.*, **99,** 4150 (1977).

(14) S. Navaratnam, B. J. Parsons, G. O. Phillips, and A. K. Davies, *J. Chem. Soc. Faraday Trans. 1*, **74,** 1811 (1978).

(15) M. R. Chedekel, E. J. Land, R. S. Sinclair, D. Tait, and T. G. Truscott, *J. Am. Chem. Soc.*, **102,** 6587 (1980).

(16) D. E. Moore and C. D. Burt, *Photochem. Photobiol.*, **34,** 431 (1981).

(17) S. P. Van and G. S. Hammond, *J. Am. Chem. Soc.*, **100,** 3895 (1978).

(18) N. J. Bunce, Y. Kumar, and L. Ravanal, *J. Med. Chem.*, **22,** 202 (1979).

ACKNOWLEDGMENTS

This work was made possible by the award of a Colombo Plan Scholarship to Swasono R. Tamat, and the generous assistance of Ciba-Geigy, Sydney.

NOTES

Evaluation of Various *N*-Substituted Azaspiranedione Derivatives as Potential Antimicrobial Agents

K. R. SCOTT *x, POLEDI G. KENNEDY *, MURRAY KEMP *, VASANT G. TELANG *, and H. W. MATTHEWS ‡

Received September 28, 1981, from the **College of Pharmacy and Pharmacal Sciences, Department of Biomedicinal Chemistry, Howard University, Washington, DC 20059*, and the ‡*Southern School of Pharmacy, Department of Biomedical Sciences, Mercer University, Atlanta, GA 30312.* Accepted for publication March 3, 1982.

Abstract □ A series of *N*-substituted hydrazines were condensed with various spiro[4.5] and [5.5]anhydrides and the resultant *N*-substituted azaspiranediones were evaluated for antimicrobial activity. None displayed any significant activity in a variety of organisms tested.

Keyphrases □ Antimicrobial agents—potential, evaluation of various *N*-substituted azaspiranedione derivatives □ Azaspiranedione—evaluation of various derivatives as potential antimicrobial agents

Previous works (1, 2) have shown a wide variety of biological effects for the azaspiro nucleus. It was of interest to extend this work to various 2-substituted-2-azaspiro-[4.5]decane-1,3-diones (I) and 3-substituted-3-azaspiro-[5.5]undecane-2,4-diones (II) and to evaluate these hydrazinoimides as potential antimicrobial agents.

It has been demonstrated (3–6) that in the fused 4-azacholestane ring various 4-alkyl-substituted derivatives

0022-3549/83/0200-0183$01.00/0

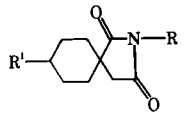
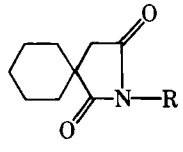


Table I—2-Substituted-2-azaspiro[4.5]decane-1,3-diones

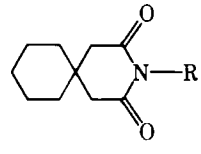
Compound	R	R¹	Yield, %	Isolation Procedure[a]	Melting Point	Boiling Point, mm	Formula	Analysis, % Calc.	Found
XIII	H	H	77	D, C	144–145° [b,c]	199–205° (1.75)	$C_9H_{13}NO_2$	C 64.64 H 7.84 N 8.38	64.82 7.82 8.40
XIV	NH_2	H	57	C	116–117° [b]	—	$C_9H_{14}N_2O_2$	C 59.30 H 7.75 N 15.38	59.59 7.80 15.40
XV	—N⬡	H	73	C	132–133° [d]	—	$C_{14}H_{22}N_2O_2$	C 67.15 H 8.86 N 11.19	67.25 8.90 11.31
XVI	—N⬡O	H	64	C	151–153° [e]	—	$C_{13}H_{20}N_2O_3$	C 61.87 H 8.00 N 11.11	61.90 8.10 11.21
XVII	NH_2	CH_3	72	D, C	130–131° [b]	109–219° (1.75)	$C_{10}H_{16}N_2O_2$	C 61.19 H 8.22 N 14.28	61.30 8.18 14.19
XVIII	—N⬡	CH_3	82	D, C	143–144° [f]	154–155° (3.75)	$C_{15}H_{24}N_2O_2$	C 68.13 H 9.16 N 10.60	68.31 9.22 10.70
XIX	—N⬡O	CH_3	79	D, C	166–167° [f]	174–184° (2.25)	$C_{14}H_{22}N_2O_3$	C 63.12 H 8.33 N 10.52	63.13 8.40 10.50

[a] Legend: D is distillation; C is chromatography (alumina, neutral; solvent benzene–methanol, 4:1). [b] Ethanol–Water. [c] Lit. (15) mp 145°. [d] Hexane. [e] Benzene. [f] Methanol.

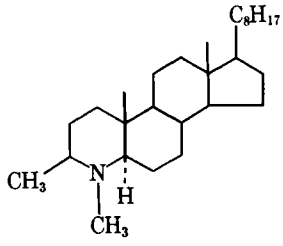
(III, IV) possessed antimicrobial activity. Thus, it was of interest to determine whether this activity could be extended to the spiro nucleus as well.



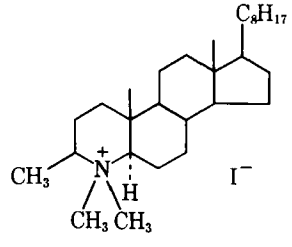
(I)



(II)

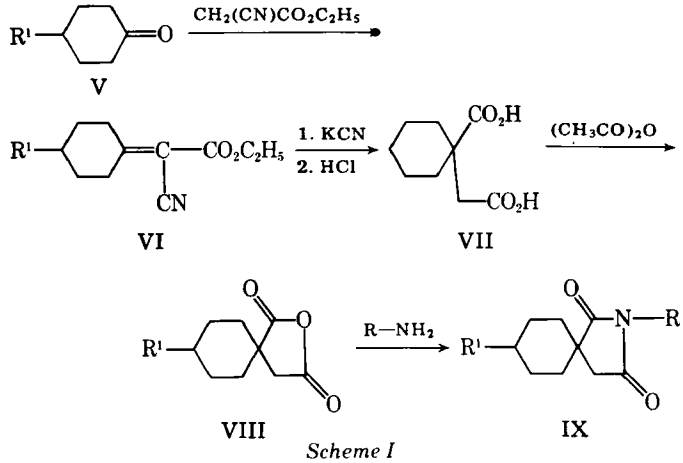


(III)



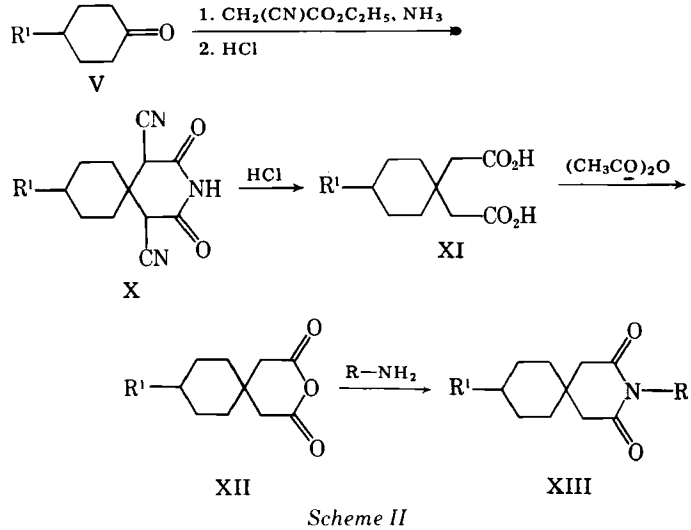
(IV)

The reaction sequence for the 2-azaspiro[4.5]decane series is shown in Scheme I:



Scheme I

The ketone (V) is subjected to a Cope procedure (7) using ammonium acetate and acetic acid as catalysts. The resultant alkylidene cyanoacetic ester (VI) was obtained in purified form on distillation. (This ester should be used within several days as decomposition is noted even under the most careful conditions.) Addition of potassium cyanide followed by acid hydrolysis produced the 1-carboxy-1-acetic acid derivative (VII), which was quantitatively converted to the anhydride (VIII), with excess acetic anhydride. Condensation of the anhydride with substituted hydrazines in the presence of a high-boiling solvent and molecular sieves followed by distillation produced the desired 2-substituted-2-azaspiro[4.5]decane (IX). The reaction sequence for the 3-azaspiro[5.5]undecane series is shown in Scheme II:



Scheme II

In this sequence, the ketone was subjected to 2.0 moles of ethyl cyanoacetate in the presence of anhydrous ammonia to yield the ammonium salt of the Guareschi imide (8) which was converted to the imide (X), on acidification

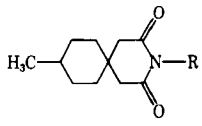


Table II—3-Substituted-3-azaspiro-9-methyl[5.5]undecane-2,4-diones

Compound	R	Yield, %	Isolation Procedure[a]	Melting Point	Boiling Point, mm	Formula	Analysis, % Calc.	Found
XX	H	82	C	161–162° [b,c]	—	$C_{11}H_{17}NO_2$	C 67.65	67.75
							H 8.78	8.89
							N 7.18	7.20
XXI	NH_2	83	C	162–163° [c]	—	$C_{11}H_{18}N_2O_2$	C 62.82	62.90
							H 8.63	8.73
							N 13.33	13.46
XXII	—N⟨⟩	73	D, C	110–111° [d]	179° [f]	$C_{16}H_{26}N_2O_2$	C 69.02	69.00
							H 9.42	9.54
							N 10.07	10.00
XXIII	—N⟨O⟩	82	C	132–133° [e]	—	$C_{15}H_{24}N_2O_3$	C 62.24	62.20
							H 8.63	8.50
							N 10.00	10.10

[a] Legend (Table I). [b] Lit. (16): 162°. [c] Methanol. [d] Benzene. [e] Hexane. [f] Ref. 1.

with hydrochloric acid. Sulfuric acid hydrolysis converted the imide into the 1,1-bisacetic acid compound (XI). Acetic anhydride, followed by hydrazine condensation as in the previous scheme, produced the anhydride (XII) and the 3-azaspiro[5.5]undecane-2,4-dione derivative (XIII), respectively. The imides were tested against a variety of microorganisms for antibacterial activity.

EXPERIMENTAL

Instruments—Melting points were obtained using a capillary melting point apparatus[1] and are uncorrected. Observed boiling points were also uncorrected. IR spectra were obtained on a spectrophotometer[2] as a Nujol mull or neat. NMR spectra were determined on a 60-MHz spectrometer[3] with tetramethylsilane as the internal reference. Elemental analyses were also performed[4].

Reagents—All ketones, ethyl cyanoacetate, and hydrazine[5], *N*-aminopiperidine, and *N*-aminomorpholine[6] were distilled before use.

Ethyl α-Cyclohexylidene-α-cyanoacetate (VI, R¹=H)—The general procedure (7) was followed using 1.0 mole of cyclohexanone and 1.0 mole of ethyl cyanoacetate with 7.7 g of ammonium acetate and 12 g of acetic acid in a 500-ml flask. Yield: 125 g (65%), bp 104–107° (0.25 mm). [lit. 150–151° (9 mm) (7), 151° (12 mm) (9), 163° (15 mm) (10), 151° (10 mm) (11–13)] IR (neat) 4.45μ (Sharp, C≡N).

Ethyl α-(4-Methylcyclohexylidene)-α-cyanoacetate (VI, R¹=CH₃)—Using the above procedure and the same molar quantities, 145 g (70%) was obtained, bp 98–104° (0.7 mm) [lit. 165–168° (14 mm), 175° (20 mm), 167° (14 mm)].

Cyclohexane-1-carboxy-1-acetic Acid (VII, R¹=H)—Using a previous procedure (9) employing 0.18 mole of ester and 0.36 mole of potassium cyanide, after solvent removal and 16 hr of refluxing in 250 ml of concentrated hydrochloric acid, 14 g (42%) of the acid was obtained, mp 132–133° (ligroin–ethyl acetate). The yield was improved to 50% by chilling the acid filtrate overnight and collecting the additional crude acid [lit. 134° (9, 14), 132° (10, 15)]. IR(Nujol) 5.75μ (Sharp, C=O).

4-Methylcyclohexane-1-carboxy-1-acetic Acid (VII, R¹=CH₃)—The same method (9) was employed on the same molar quantities of ester and cyanide as with compound VII above. Yield: 19 g (53%) mp 173–174° (ligroin–ethyl acetate).

Anal.—Calc. for $C_{10}H_{16}O_4$: C, 59.97, H, 8.00. Found: C, 59.73, H, 7.90.

Cyclohexane-1-carboxy-1-acetic Acid Anhydride (VIII, R¹=H)—Using a previous procedure (9), from 0.05 mole of acid 8.1 g (90%) of anhydride was obtained, bp 112° (1.75 mm), which slowly solidified on standing, mp 57° [lit. 56° (9), 57° (14)]. IR(Nujol) 5.50μ (Sharp, C=O).

4-Methylcyclohexane-1-carboxy-1-acetic Acid Anhydride (VIII, R¹=CH₃)—As previously indicated, the title compound was quantitatively prepared, bp 124–126° (3 mm), solidifying on standing, mp 60–61°.

Anal.—Calc. for $C_{10}H_{14}O_3$: C 65.90; H, 7.76. Found: C, 65.90; H, 7.64.

The Guareschi Imide-9-methyl-1,5-dicyano-3-azaspiro[5.5]-undecane-2,4-dione (X, R¹=CH₃)—A general procedure (16) was modified using 1.0 mole of ketone and 2.0 moles of ethyl cyanoacetate in a 1.5-liter thick-wall flask. After cooling to −5°, 400 ml of absolute ethanol saturated with anhydrous ammonia and cooled to −5° was added, the flask stoppered, taped securely, and stored at 0° for 1 week. The crude ammonium salt of the Guareschi imide (8, 17) was filtered, washed with alcohol and then ether, and air dried. The product was dissolved in 1 liter of boiling water, filtered, and the hot solution acidified with excess concentrated hydrochloric acid. The product, on cooling, was filtered, washed with water, and dried at 100° to give 115 g (47%), mp 217–218° (methanol) [lit. 217–218° (16)].

4-Methylcyclohexane-1,1-diacetic Acid (XI, R¹=CH₃)—Using a previous procedure (16), 0.5 mole of imide, 240 ml of concentrated sulfuric acid, which after standing overnight at room temperature, was diluted with 225 ml of water and refluxed for 24 hr, resulting in a product, 140 g (65%), mp 157–158° [lit. 158° (16)].

4-Methylcyclohexane-1,1-diacetic Acid Anhydride (XII, R¹=CH₃)—The previous procedure produced the desired product, bp 152–154° (1.25 mm), which solidified on standing, mp 53° [lit. bp 212° (20 mm), mp 53° (16)].

General Procedure for the Preparation of Azaspiranediones—The anhydride, 0.05 mole, and the substituted hydrazine were added to 10 ml of xylene (distilled from sodium) in a 50-ml flask containing molecular sieves[7]. The mixture was stirred magnetically and refluxed for 2–8 hr. The product precipitated on cooling overnight, and after separation, the residual oil was seeded and chilled. The entire residue was combined and either chromatographed or distilled. The distillate was then either chromatographed or recrystallized (Tables I and II). As previously reported (2), azaspiro[5.5]undecane-2,4-diones formed unstable hydrochloride salts. This was also observed with the undecanes synthesized in this study as well as the azaspiro[4.5]decane-1,3-diones.

RESULTS AND DISCUSSION

In addition to the potential antimicrobial activity of various *N*-substituted azasteroids (3–6), adamantane spiro compounds (18, 19) have been evaluated for antiviral activity and spirofluorene (20) and spiroperimidines (21) have been tested for antineoplastic activity. Compounds XIV–XVI and XXI–XXIII were thus submitted for antimicrobial screening. The analyses were performed by the use of the Kirby–Bauer technique (22) with the following organisms: *Escherichia coli*, *Staphylococcus aureus*, *Sarcina lutea*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* (23). The compounds showed no activity at concentrations up to 100 μm/ml.

[1] Thomas-Hoover.
[2] Beckman IR-18A.
[3] Varian EM 360A.
[4] Performed by the Schwarzkopf Microanalytical Laboratory, Woodside, NY 11377.
[5] Eastman Chemicals.
[6] Columbia Organics, Inc.
[7] Molecular sieves, type 4A, Fisher Scientific Co.

REFERENCES

(1) L. M. Rice, C. F. Geschickter, and C. H. Grogan, *J. Med. Chem.* **6**, 388 (1963).

(2) C. H. Grogan, C. F. Geschickter, and L. M. Rice, *ibid.*, **7**, 78 (1964).

(3) R. F. Smith, D. E. Shay, and N. J. Doorenbos, *J. Bacteriol.*, **85**, 1295 (1963).

(4) R. F. Smith, D. E. Shay, and N. J. Doorenbos, *Proc. Pa. Acad. Sci.*, **36**, 113 (1962).

(5) R. F. Smith, D. E. Shay, and N. J. Doorenbos, *Appl. Microbiol.*, **11**, 542 (1963).

(6) R. F. Smith, D. E. Shay, and N. J. Doorenbos, *J. Pharm. Sci.*, **53**, 1214 (1964).

(7) A. C. Cope, C. M. Hofmann, C. Wyckoff, and E. Hardenbergh, *J. Am. Chem. Soc.*, **63**, 3452 (1941).

(8) I. Guareschi, *Atti. Accad. Sci. Torino*, **36**, 443 (1900/1901).

(9) I. Vogel, *J. Chem. Soc.*, **1928**, 2010.

(10) A. Lapworth and J. A. McRae, *ibid.*, **121**, 2741 (1922).

(11) S. F. Birch, G. A. R. Kon, and W. S. G. P. Norris, *ibid.*, **123**, 1361 (1923).

(12) V. J. Harding, W. N. Haworth, and W. H. Perkin, *ibid.*, **93**, 1943 (1908).

(13) A. C. Cope, *J. Am. Chem. Soc.*, **59**, 2327 (1937).

(14) W. S. G. P. Norris and J. F. Thorpe, *J. Chem. Soc.*, **119**, 1199 (1921).

(15) F. Dickens, L. Horton, and J. F. Thorpe, *ibid.*, **124**, 1830 (1924).

(16) J. F. Thorpe and A. S. Wood, *ibid.*, **103**, 1586 (1913).

(17) G. A. R. Kon and J. F. Thorpe, *ibid.*, **115**, 693 (1919).

(18) K. Lundahl, J. Schut, J. L. M. A. Schlatmann, G. B. Paerels, and A. Peters, *J. Med. Chem.*, **15**, 129 (1972).

(19) R. VanHes, A. Smit, T. Kralt, and A. Peters, *ibid.*, **15**, 132 (1972).

(20) H.-L. Pan and T. L. Fletcher, *ibid.*, **10**, 957 (1967).

(21) W. Wasulko, A. C. Noble, and F. D. Popp, *ibid.*, **9**, 599 (1966).

(22) A. W. Bauer, W. M. M. Kirby, J. C. Sherris, and M. Turck, *Am. J. Clin. Pathol.*, **45**, 493 (1966).

(23) D. Perlman, K. L. Perlman, M. Bodanszky, A. Bodanszky, R. L. Foltz, and H. W. Matthews, *Bioorgan. Chem.*, **6**, 263 (1977).

ACKNOWLEDGMENTS

The authors wish to thank Dr. Peter E. Frankenberg and Mr. C. H. Roberts of the Du Pont Company, Wilmington, Del., for the NMR analyses.

Dissolution Apparatus for Gels

HO-LUN WENG and EUGENE L. PARROTT ˣ

Received January 11, 1982, from the *Division of Pharmaceutics, College of Pharmacy, University of Iowa, Iowa City, IA 52242.* Accepted for publication March 11, 1982.

Abstract □ A modification of the USP Dissolution Apparatus 2 is presented for use in the measurement of the release of a medicinal compound from a pharmaceutical gel.

Keyphrases □ Dissolution—apparatus for gels, modification of the USP Dissolution Apparatus 2 □ Pharmaceutical gels—dissolution apparatus, modification of the USP Dissolution Apparatus 2

The *in vitro* test methods for measuring the dissolution of a medicinal compound from a tablet or a capsule are defined in the United States Pharmacopeia (USP) (1). Mathur *et al.* (2) appear to be the first to have reported *in vitro* dissolution testing of suspensions. It seems that dissolution testing of any dosage form that may restrict the delivery of molecules of the medicinal compound to the GI epithelium is advisable.

This report presents a modification of the USP Dissolution Apparatus 2, that can be used to measure dissolution profiles from pharmaceutical gels.

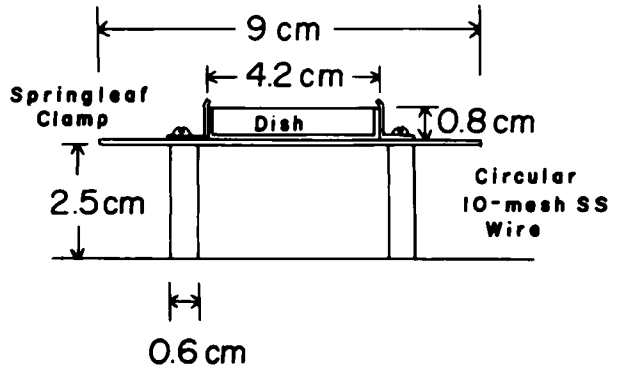


Figure 1—*Modification of the USP Dissolution Apparatus 2 for gels.*

EXPERIMENTAL

Dissolution Apparatus—A circular, 10-mesh stainless-steel cloth with a 9.0-cm diameter was fitted with four 2.5-cm plastic legs (diameter 0.6 cm) so that, as it rested on the bottom of the reaction vessel, the wire cloth was at the point of curvature of the base of the vessel. Four springleaf clamps were affixed to the upper surface of the wire cloth to hold the glass dish in the center of the platform (Fig. 1). The dish was cut from a 50-ml beaker and had 3.9-cm i.d. and an outer height of 0.8 cm.

A 5-g sample of the gel was weighed in the dish on an analytical balance, and using forceps the dish was gently lowered through the dissolution medium in the assembled apparatus and was inserted into the clamps. The time required by an experienced operator was ~30 sec. With thin gels, the level spontaneously adjusted to the horizontal plane; with very viscous gels, the surface may be leveled by use of a spatula prior to weighing. In the gels investigated, the 5-g samples completely filled the dish to the rim. If a particular gel had a high density, it would be advisable to use a greater weight, which would fill the dish. The dissolution medium was 900 ml of distilled water or 0.1 *N* HCl at 37 ± 0.5°. The paddle of the modified USP Dissolution Apparatus 2 was centrally positioned 2.5 cm above the rim of the dish. The apparatus was then started. Samples were withdrawn by a pipet through a glass filter. Ephedrine sulfate was assayed using a previously described method (3).

Dosage Forms—Tablets containing 25 mg of ephedrine sulfate was prepared by direct compression in a single-punch tablet machine. A portion of the batch was compressed to a hardness of 4 kg; the remainder was compressed to an 8-kg hardness. The formulation was:

Ephedrine sulfate[1]	25 mg
Microcrystalline cellulose[2]	190 mg
Lactose[3]	382 mg
Magnesium stearate[4]	3 mg

[1] USP.
[2] Avicel PH 101.
[3] USP, spray dried.
[4] NF.

0022-3549/83/0200-0186$01.00/0